Identification of key genes and long non-coding RNA expression profiles in osteoporosis with rheumatoid arthritis based on bioinformatics analysis

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Research article

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Abstract

Background

Rheumatoid arthritis (RA) is a chronic systemic tissue disease characterized by osteoporosis and fragility fractures. However, the molecular mechanisms remain unclear. The aim of this study was to identify differentially expressed genes, associated biological processes and long non-coding RNAs (lncRNAs) between osteoporosis and RA using a bioinformatics approach to elucidate the pathogenesis.

Results

A total of 28 mRNAs and 2 lncRNAs were differentially expressed both in RA and osteoporosis. Chromosome mapping of consensus DEmRNAs revealed that chromosome 1 contained the greatest number. GO and KEGG results suggested that the DEmRNAs in RA and osteoporosis were commonly involved in “platelet degranulation”,“platelet alpha granule”, “platelet activation”, “tight junction” and “leukocyte transendothelial migration”. Most of them were related to PLTs. In the PPI network, MT-ATP6 and PTGS1 might be the hub genes. Interestingly, MT-ATP6 derived from mitochondrial DNA. Through co-expression analysis, we identify two potential key pairs of lncRNAs and target mRNAs, including RP11 – 815J21.2 - MT – ATP6 and RP11 – 815J21.2- PTGS1. It indicated that lncRNA RP11 – 815J21.2 could be a potential candidate as biomarker.

Conclusions

Analysis of this study revealed that dysregulated lncRNAs and mRNAs in RA and osteoporosis might affect the platelet activation and some associated pathways. MT-ATP6 and PTGS1 might be a novel target. The biological function of lncRNA RP11 – 815J21.2 need to be further validated.

Background

Rheumatoid arthritis (RA) is a systemic autoimmune disease with unknown etiology characterized by progressive and systemic inflammation, resulting in joint destruction and extra-articular manifestations. In addition to periarticular bone loss and local erosions, bone loss begins early during the disease. Due to inflammation-induced osteoclast activation, RA has been identified as a critical risk factor for osteoporosis [1]. Osteoporosis is one of the most common diseases of the skeletal system that reduces bone density and changes bone structure [2], which is why the bones become more brittle. So the risk of vertebral and non-vertebral fracture is increasing [3]. Since osteoporotic fracture can cause progressive pain, and induce significant morbidity and mortality as well as increased costs [4], the treatment of osteoporosis to minimize fracture risk in patients with RA is important.

Osteoclast activation is regulated by lots of factors including growth factors and hormones[5]. It has established that the receptor activator of the nuclear factor-κB ligand(RANKL) and macrophage colony-stimulating factor (M-CSF) are involved in osteoclast differentiation[6]. Meanwhile, some inflammatory cytokines, such as the TNF-α, IL-1,IL-6 and IL-17 can upregulate the RANKL[7]. So, at the molecular level, a common feature between RA and osteoporosis may be the same change in gene expression. Furthermore, lncRNAs are hypothesized to be involved in the differentiation and activation of CD4 + T cells[8], and they are also reported to act as both osteogenic differentiation inhibitor and promotor[9].

This pilot study aimed to identify aberrantly expressed mRNAs and lncRNAs profile between RA and osteoporosis and explore their potential function. In particular, using public databases, we identified the clinical significance of lncRNAs, it may be useful as diagnostic and prognostic biomarkers and provide novel therapeutic signaling targets in related to osteoporosis in RA patients.

Results

Principal component analysis verifying independence of each group.

To distinguish the significant difference between samples, PCA was performed. The results showed that RA samples VS. control samples in GSE94519 dataset and osteoporosis samples VS. control samples in GSE152293 dataset displayed a significant difference. The vertical axis stands for the contribution rate for each principal component. The number of principal components is on the horizontal axis (Fig.1A). The cumulative contribution rates of PCA1 and PCA2 of the two datasets are 61.4% and 62.6% respectively. It indicated that the first two components were enough to separate the two groups, and each group is independent of each other (Fig.1B).

Differentially expressed mRNAs and lncRNAs in RA and osteoporosis.

Aberrantly expressed mRNAs and lncRNAs associated with RA and osteoporosis were identified based on the criteria: $|\log_2 FC|>1$ and $p<0.05$. Volcano plots were used to visualize the differentially expressed mRNAs and lncRNAs (Fig.2). In RA, a total of 649 differentially expressed mRNAs and 313 lncRNAs were identified, of which 385 mRNAs and 89 lncRNAs were upregulated and the remaining ones were downregulated. Then, 535 mRNAs displayed differential expression in osteoporosis, including 227 upregulated mRNAs and 308 down regulated mRNAs. Of 33 lncRNAs that showed differential expression ,7 were upregulated and 26 were downregulated.

Go and pathway analysis from each disease group.

Until now, the functions of most lncRNAs have not been well annotated. Therefore, the role that lncRNAs play may be forecasted by analyzing differentially expressed mRNAs. To gain insights into the biological roles of DEmRNAs from RA and osteoporosis versus control samples, we performed GO enrichment

analysis. The GO results including biological process, cell component and molecular function (Fig.3 A,C). KEGG pathway analysis was used to understand the signaling pathway enrichment of DEmRNAs. The results were shown in Fig.3 B,D. Furthermore, some DEmRNAs from the RA group and osteoporosis group respectively were commonly enriched in the same GO terms which were platelet degranulation (BP) and platelet alpha granule (CC) (Table 1). The KEGG pathways indicated that certain DEmRNAs screened from the two diseases were equal to associate with the platelet activation, tight junction and leukocyte transendothelial migration (Table 2), and the platelet activation exhibited highly significant enrichment between RA and osteoporosis.

Table 1. Common GO terms between RA and osteoporosis

	Differentially expres
GO term and function	RA
GO:0002576-platelet degranulation	PF4,PPBP,F13A1,LY6G6F,GTPBP2,CLU,LYN,TLN1,SRGN,VCL,FERMT3,CTSW,CD9,ABCC4,VTI1B,ALDOA,ITGB3,RAB2
GO:0031091-platelet alpha granule	PF4,PPBP,SNCA,F13A1,LY6G6F,TREML1,GTPBP2,CLU,SRGN,FERMT3,CD9,VTI1B,ALDOA,ITGB3,TMSB4X,TIMP1,VAM

RA: rheumatoid arthritis, GO: gene ontology

Table 2. Common KEGG pathways between RA and osteoporosis

	Differ
KEGG ID and pathways	RA
hsa04611-platelet activation	FCGR2A,AKT3,ACTB,LYN,TLN1,PTGS1,ITGB1,FERMT3,GP1BA,ITGB3,GNA13,MYL12A,MYL12B,GNAS,GP1BB,MAPI
hsa04530-tight junction	CDC42,CLDN22,RAPGEF6,ACTB,CLDN4,CLDN15,ITGB1,AMOTL1,TUBA1B,TUBA1C,MYL6,CLDN10,MYL12A,MYL12
hsa04670-leukocyte transendothelial migration	CDC42,MYL5,CLDN22,CTNNA1,ACTB,CLDN4,CLDN15,ITGB1,VCL,CLDN10,MYL12A,MYL12B,MYL9

RA: rheumatoid arthritis, KEGG: the Kyoto Encyclopedia of Genes and Genomes

Common DEmRNAs and DElncRNAs both in RA and osteoporosis.

We comprehensively analyzed the DEmRNAs and DElncRNAs in RA and osteoporosis. A total of 28 mRNAs were differentially expressed both in RA and osteoporosis, among which 23 mRNAs were upregulated and 5 mRNAs were downregulated. Furthermore, 2 common lncRNAs (RP11-815J21.2 and CTD-3092A11.2) were screened from the two datasets. These were illustrated by the Venn diagram (Fig. 4A). In addition, the heatmap depicted the difference in expression pattern of the common DEmRNAs (Fig. 4B). Chromosome mapping of consensus DEmRNAs revealed chromosome distribution (Fig. 4C), with chromosome 1 containing the greatest number. While two genes on the X chromosome showed difference, there was no gene affected on the Y chromosome.

PPI network of common DEmRNAs and hub genes identification

To further explore the relationships between the common DEmRNAs at the protein level, the 28 common DEmRNAs were analyzed by STRING. With the predefined criterion of interaction score> 0.4, 17 of the 28 common DEmRNAs were included in the PPI network, and there were 37 nodes and 95 edges screened to establish the PPI network (Fig. 5A). In the network, the top 12 key genes according to the MCC method were selected using the CytoHubba plugin (Fig. 5B). The MT-ATP6 and PTGS1 might be the hub genes and essential for RA and osteoporosis.

lncRNA-mRNA co-expression network Analysis

The lncRNA-mRNA co-expression network was constructed based on the correlation analysis between the common lncRNAs and mRNAs. P-value<0.05 and coefficient>0.6 were set as the criteria to predict lncRNA-mRNA pairs. Functional prediction of lncRNAs was performed based on the co-expressed mRNAs. The network could be used to identify candidate biomarkers. As shown in Fig.6 A, it consisted of 23 nodes and 23 connections among 21 DEmRNAs and 2 DElncRNAs. There were 1 negative and 22 positive interactions within the network. Moreover, we then plotted the expression level of the two lncRNA-mRNA pairs of RP11−815J21.2 - MT−ATP6 and RP11−815J21.2- PTGS1 in these two dataset and found they were upregulated in RA and osteoporosis (Fig.6 B). The two mRNAs were predicted essential in PPI network and RP11−815J21.2 might have potential roles in regulating the expression of them.

Discussion

The pathophysiology of RA is complex, perpetuated by an array of cells whose functions have been altered and converted to autoimmune cells, or made to follow an inflammatory pathway [10]. As an autoimmune disease, the reactivity of immune cells towards articular cells is central to RA. Neutrophils, lymphocytes, and platelets have been reported to play a role in the control of inflammation and are associated with alterations in secondary to inflammation [11]. Meanwhile, pro-inflammatory molecules are usually present in the circulation of patients with RA and contribute to the immunopathogenesis. These uncontrolled molecules are also closely linked to abnormal blood clotting via increased oxidative stress that leads to changes in cells of haematological system (including platelets) [12]. Research has showed that platelets and platelet-derived microparticles (MPs) are involved in the inflammatory processes and are found in arthritic joints [13]. Their activation is regulated by platelet signaling and secreted proteins, miRNAs, and molecular pathways. Then, they release cytokines, chemokines and growth factor stored in α-granules and dense granules to mediate their main functions, support the recruitment of inflammatory cells to tissue sites and trigger inflammation [14, 15].

The platelets (PLTs) arise in the bone marrow from megakaryocytes, some studies found that platelets have a significant role in skeletal homeostasis, modulating bone formation and resorption [16]. Many studies indicated the supportive effect of PLTs on bone formation result from platelet-derived growth factors (PDGFs) which favor bone formation by affecting cell proliferation, chemotaxis differentiation, and extracellular matrix synthesis [17]. However, the exact function of PLTs and its MPs on bone resorption is still complex to understand. There are several preclinical and clinical researches showing the role of PLTs in osteoclastogenesis and bone resorption. PLTs have vitamin D receptors which are less expressed in osteoporosis patients and related to the variation of BMD [18]. Eroglu, S. et al. found that the PLT/lymphocyte ratio also correlated with low BMD [19]. The mediators released from PLTs may be involved in bone remodeling. EGF cooperating with RNAKL with osteoclasts and TGF-B stimulating OPG synthesis are more likely to enhance the bone resorption. Moreover, TXA2 induces osteoclastogenesis and enhances bone resorption [17, 20]. Chronic inflammation may play a pivotal role in osteoporosis and activated platelet [21]. Pro-inflammatory cytokines enhance oxidative stress which contributes to platelet activation. On one hand, those cytokines promote osteoclast formation and simulate bone resorption. On the other hand, activated PLTs affect osteoclastogenesis through prostaglandin and RANKL signaling [22].

In the current study, we identified 385 up-regulated and 264 down-regulated DEmRNAs between RA and control samples using bioinformatics analysis. Similarly, a total of 535 DEmRNAs including 227 up- and 308 down-regulated were identified between osteoporosis and control samples. As cumulative evidence has shown that co-expressed genes normally represent those with similar expression profiles that also frequently participate in similar biological processes [23], we further performed GO and pathway enrichment analyses. These analyses suggested that the DEmRNAs in RA and osteoporosis were commonly involved in “platelet degranulation”,” platelet alpha granule”,” platelet activation”,” tight junction” and “leukocyte transendothelial migration”. Most of them were related to PLTs, and the results were consistent with what has been discussed above. Furthermore, to identify common genes both in RA and osteoporosis, each DEmRNAs in them were intersected. A total of 28 common DEmRNAs including 23 up-regulated and 5 down-regulated were identified. Heatmap provided sufficient evidence indicating the presence of differentially expressed mRNAs. Chromosome mapping showed the distribution of genes in chromosome. Previously studies confirmed that X-chromosome consisted of many genes which were associated with RA and osteoporosis. Like CD99, IRAK-1, LAMP-2, CD40L,TLR7,DDX3X, XIAP and USP27X,they are involved in autoimmunity [24]. Van Dijk FS.et al. found that mutation in PLS3 might result in X-linked osteoporosis [25]. Our results indicated that the dysregulation of SH3BGRL and TMSB4X on X chromosome may be associated with RA and osteoporosis. Meantime, some studies indicated that the genes in chromosomes 1 and 13 may be related to RA and osteoporosis [26, 27, 28]. Our results showed chromosome 1 containing the greatest number of dysregulated genes.

The subsequent construction of the PPI network using the common DEmRNAs identified 17 genes as potential key genes involved in RA and osteoporosis. PTGS1 was identified as one of the hub genes and has been connected with multiple pathological disorders including inflammation, arthritis and cancer [29]. Upregulation of the PTGS1 and PTGS2 pathways of arachidonic acid is thought to be involved in the development of rheumatic diseases [30]. And another study in both ex vivo and in vivo showed that PTGS1 which controlled osteogenesis of adipose-derived stem cells was involved in the osteogenic differentiation [31]. Cho HW et al. reported that PTGS1 was associated with osteoporosis [29]. In our study, PTGS1 was over-expressed and also enriched in platelet activation pathway both in RA and osteoporosis, indicating its potential role in pathogenesis. In turn, MT-ATP6, another hub gene, derives from mitochondrial DNA. Previous studies have confirmed that each mitochondrion has multiple copies of mitochondrial DNA (mtDNA) which encode 13 protein subunits of the electron transport chain and 22 tRNAs and 2rRNAs [32]. In the plasma and synovial fluid of RA patients, mtDNA levels are higher than in control subjects. Moreover, they observed that mtDNA induces TNF-α expression through NF-κB activation, participating in inflammation and tissue injury [33]. Juping Du et al. found that enrichment of variants in MT-ATP6 was detected in RA patients [34]. In our study, MT-ATP6 was enriched both in RA and osteoporosis. Interestingly, another mitochondrial DNA A3243G mutation in blood leukocytes were found to be significantly associated with lower bone mineral density [35]. Further evidence for mitochondrial dysfunction as a potential contributor to osteoporosis is seen in mice with a mitochondrial transcription factor A(TFAM) knockout specific to osteoclasts [36]. So the accumulating mtDNA mutations may play a significant role in RA and osteoporosis.

In recent years, lncRNA related research has attracted the attention of various fields. However, lncRNAs have just begun to be understood, and the majority of them have not yet been researched. To explore the biological functions of lncRNAs, we undertook a comprehensive analysis and employed an co-expression network to identify interactions based on the 2 common differentially expressed lncRNAs and the 21 common differentially expressed mRNAs. Importantly, we identify two potential key pairs of lncRNAs and target mRNAs, including RP11 – 815J21.2 - MT – ATP6 and RP11 – 815J21.2- PTGS1. Prior study had showed that MT – ATP6 and PTGS1 were related to RA and osteoporosis, and we found the two pairs were significantly higher than those of control. But up to now, there is no report on this novel lncRNA in any disease, and our work filled the gap of lncRNA RP11 – 815J21.2. The result suggests that lncRNA RP11 – 815J21.2 could be a potential candidate as biomarker and need to be further evaluated and investigated using advanced diagnostic methods and more samples.

Conclusion

In summary, our study identified a set of co-differentially expressed mRNAs and lncRANs both in RA and osteoporosis. Based on these, we performed a series of analyses, which may contribute to the finding of molecular mechanisms. The lncRNA identified in our constructed co-expression network may have an important impact on patients and can serve as new diagnostic biomarker, prognostic factor.

Materials And Methods

Data resources

GSE94519 and GSE152293 datasets were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The microarray dataset GSE94519 contained data from 6 samples, including 3 RA and 3 control samples. It was based on the GPL20115 platform. The RNA sequence dataset GSE152293 contained data from 3 osteoporosis and 3 control samples, which was produced using an Illumina HiSeq 2000 (GPL11154). Principal component analysis (PCA) was done for the datasets for dimensionality reduction and quality control.

Screening of differentially expressed mRNAs (DEmRNAs) and long non-coding RNAs (DElncRNAs)

Probes in GSE94519 data file were annotated based on the platform annotation file. Those without matching gene symbols were removed. In instances where different probes mapped to the same gene, the values of probes corresponding to the same gene were averaged and defined as the final expression value. The FPKM values downloaded from GSE152293 dataset were used to evaluate the expression level of mRNAs and lncRNAs. We identified mRNAs and lncRNAs by using the Ensembl database (http://www.ensembl.org/ index.html, version 104) [37] and the platform annotation file. Those that were not included in the database or the platform file were excluded. Then, we performed the differential analysis in the R computing environment using limma package [38]. The mRNAs and lncRNAs with $|\log_2 FC|>1$ and p-value<0.05 were screened as DEmRNAs and DElncRNAs.

For the obtained differentially expressed mRNAs and lncRNAs, we generated volcano maps using the gplots in the R platform. DEmRNAs and DElncRNAs overlapping between datasets were obtained using vennDiagram package [39].

Functional enrichment analysis

Functional enrichment analysis of DEmRNAs was performed in R using the function of clusterProfiler [40]. Gene ontology (GO) is a commonly used bioinformatics tool that provides comprehensive information on gene function, consists of molecular functions (MF), biological processes (BP), and cellular components (CC). KEGG pathway enrichment analysis was used to find the pathway terms. P<.05 was used as the cutoff criterion for the functional enrichment analysis.

Circular visualization of the common DEmRNAs

To help us have a better view of the common differentially expressed mRNAs including their gene symbols and chromosomal locations. RCircos package in R was used for our data presenting [41].

Construction of the protein–protein interaction (PPI) network and analysis of hub genes

PPI network was constructed by the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/), a database to analyze the connections among the proteins of differentially expressed genes [42]. The common DEmRNAs were submitted to the Search Tool. The results with a minimum interaction score of 0.4 were visualized using Cytoscape software [43]. Furthermore, CytoHuba, a Cytoscape plugin app, was utilized to explore the hub genes in the PPI network.

lncRNA-mRNA co-expression network

We used batch correction to obtain a consensus of common DEmRNAs and DElncRNAs between the two datasets, then the data were normalized. R function cor.test was used to compute Pearson correlation coefficient between lncRNAs and mRNAs. Pearson correlation coefficient>0.6 and p<0.05 were selected as thresholds and a lncRNA-mRNA network was plotted by Cytoscape.

Abbreviations

GEO: Gene Expression Omnibus; PCA: Principal component analysis; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes; MF: molecular functions; BP: biological processes ;CC: cellular components ; PDGF: Platelet-Derived Growth Factor; BMD: Bone mineral density; EGF: Epidermal Growth Factor; STING: Stimulator of Interferon Genes; PPI: Protein-Protein Interaction; RNAKL: Receptor Activator of Nuclear Factor-κ B Ligand; TGF: Transforming growth factor; TXA2: Thromboxane A2.

Declarations

Acknowledgements

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Author Contributions Jinyu An conceived the idea and designed this study. Huilong Wen collected and prepared the data. Huidong Hu and Yixiong Wu helped check the data. Xingna Ma aided in revising the manuscript and were responsible and accountable for the accuracy or integrity of the work.

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Availability of data and materials

The datasets presented in this study can be found at NCBI GEO. (GSE94519: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE94519. GSE152293: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE152293.)

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflict interests.

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Figures

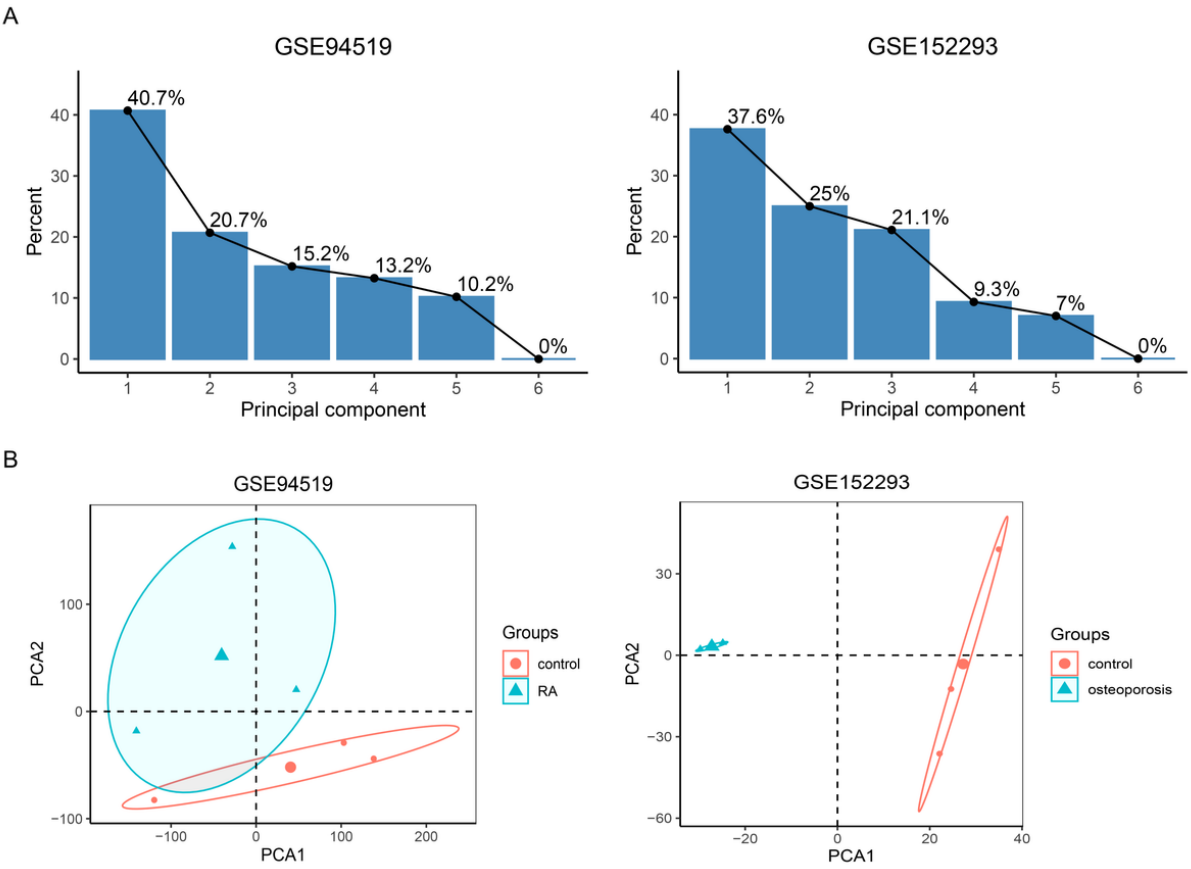

Figure 1. Results from the principal component analysis.(A) Bar plots showing the proportion of variance evaluated for the two datasets. (B) Two-dimensional plots with the top two principal components. Horizontal and vertical axes represent the distribution of each sample with PCA1 and PCA2.

Figure 1

See figure for legend.

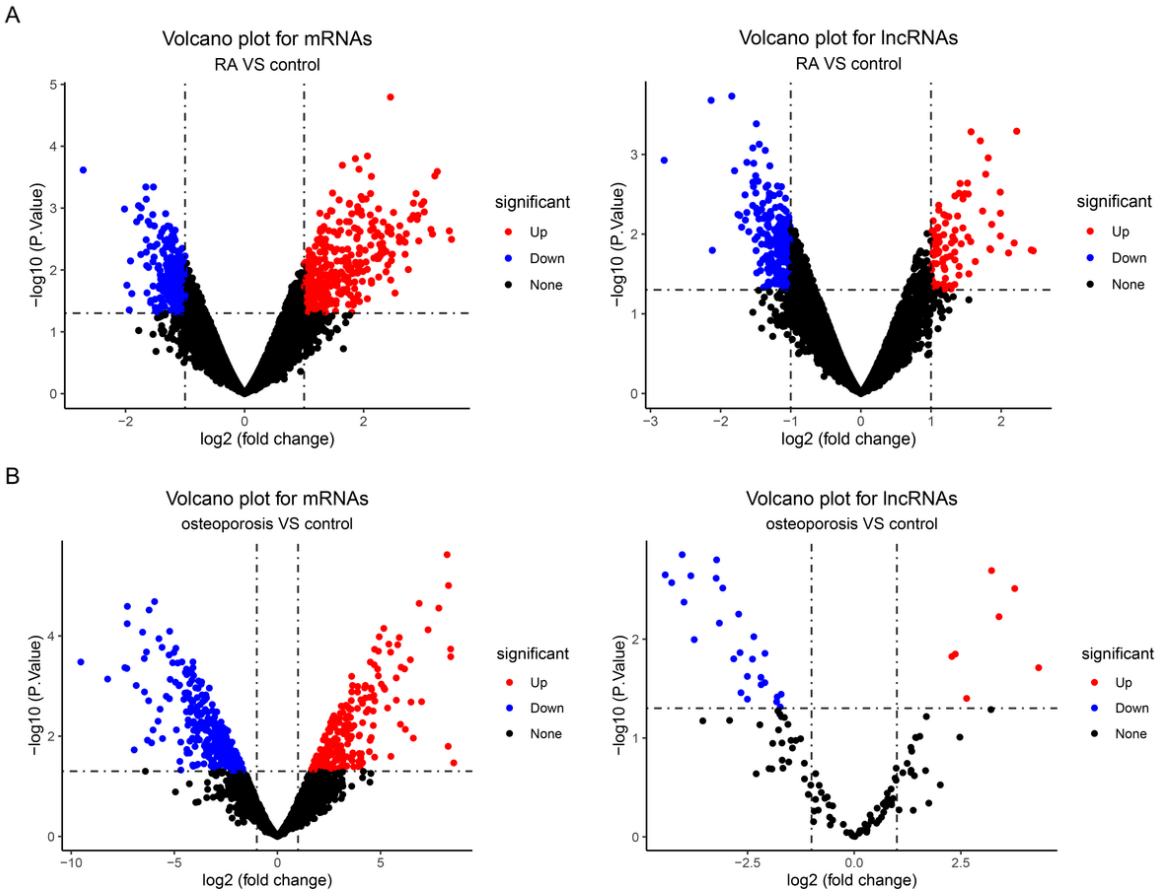

Figure 2. Volcano plots of differentially expressed mRNAs and lncRNAs in RA vs. control samples (A) and osteoporosis vs. control samples (B). Up-regulated mRNAs and lncRNAs (FC>1,p<0.05) are labeled red , whereas down regulated mRNAs and lncRNAs (FC<-1,p<0.05) are labeled blue.

Figure 2

See figure for legend.

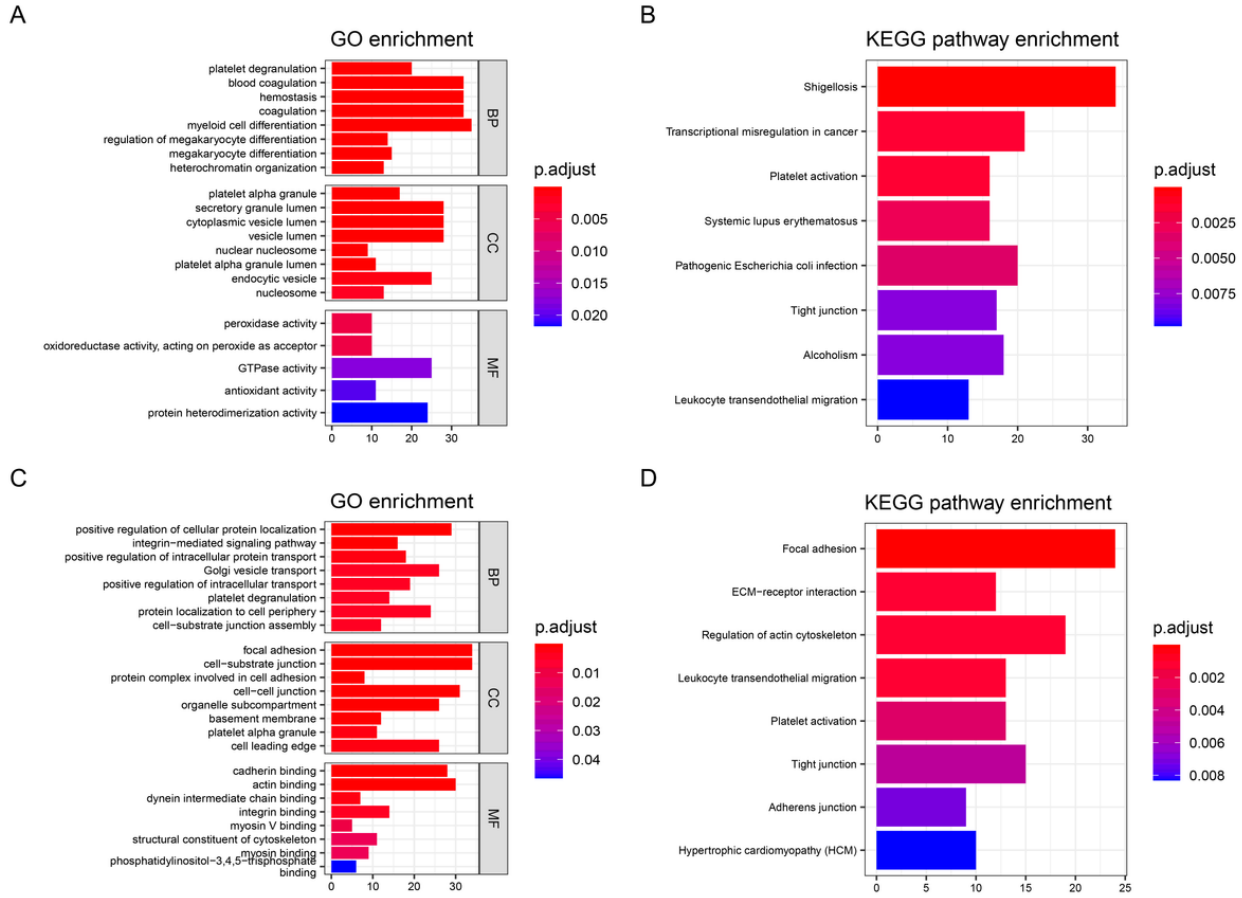

Figure 3. GO enrichment analysis and KEGG pathways of the differentially expressed mRNAs in RA vs. control samples (A,B) and osteoporosis vs. control samples (C,D). GO= gene ontology, CC=cellular component, MF= molecular function, BP=biological process, KEGG=kyoto encyclopedia of genes and genomes.

Figure 3

See figure for legend.

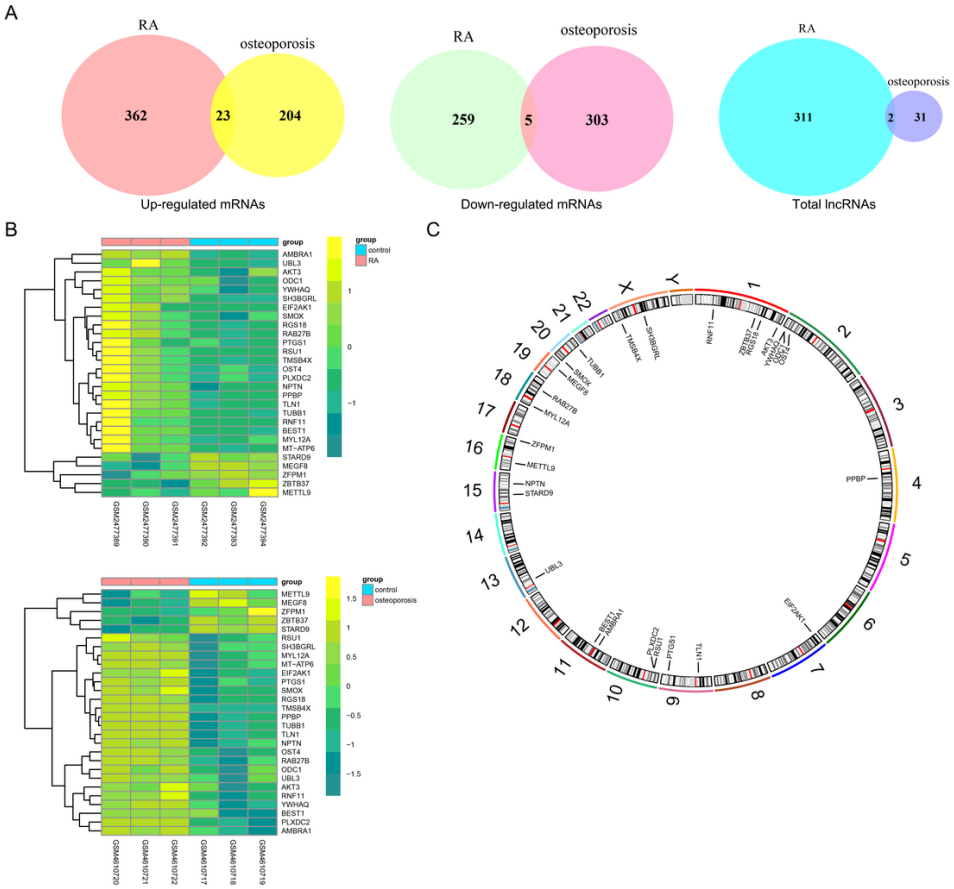

Figure 4. Analysis of common DEmRNAs and DElncRNAs both in RA and osteoporosis. (A) Venn diagram demonstrates the intersections of DEmRNAs and DElncRNAs between RA and osteoporosis. (B) Heatmap of common DEmRNAs coloring the samples-groups in RA and osteoporosis. (C) Chromosome mapping of consensus DEmRNAs.

Figure 4

See figure for legend.

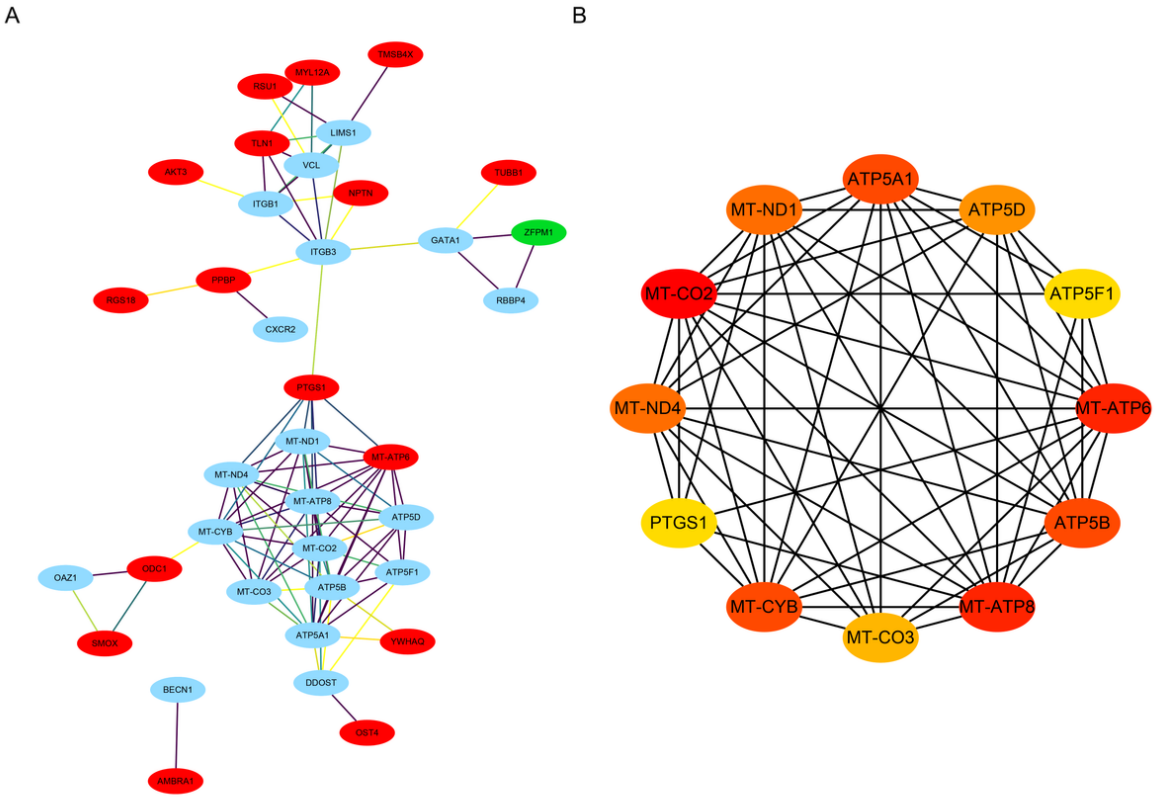

Figure 5. Protein–protein interaction (PPI) network. (A) Using the STRING, a total of 17 of common DEmRNAs (16 up-regulated genes shown in red and one down-regulated genes shown in green) were filtered into a PPI network. A darker colored line between two proteins means a greater interaction.(B) The hub genes network. The top 12 genes derived from the MMC method were selected with CytoHubba plugin. Advanced ranking is reflected by a redder color.

Figure 5

See figure for legend.

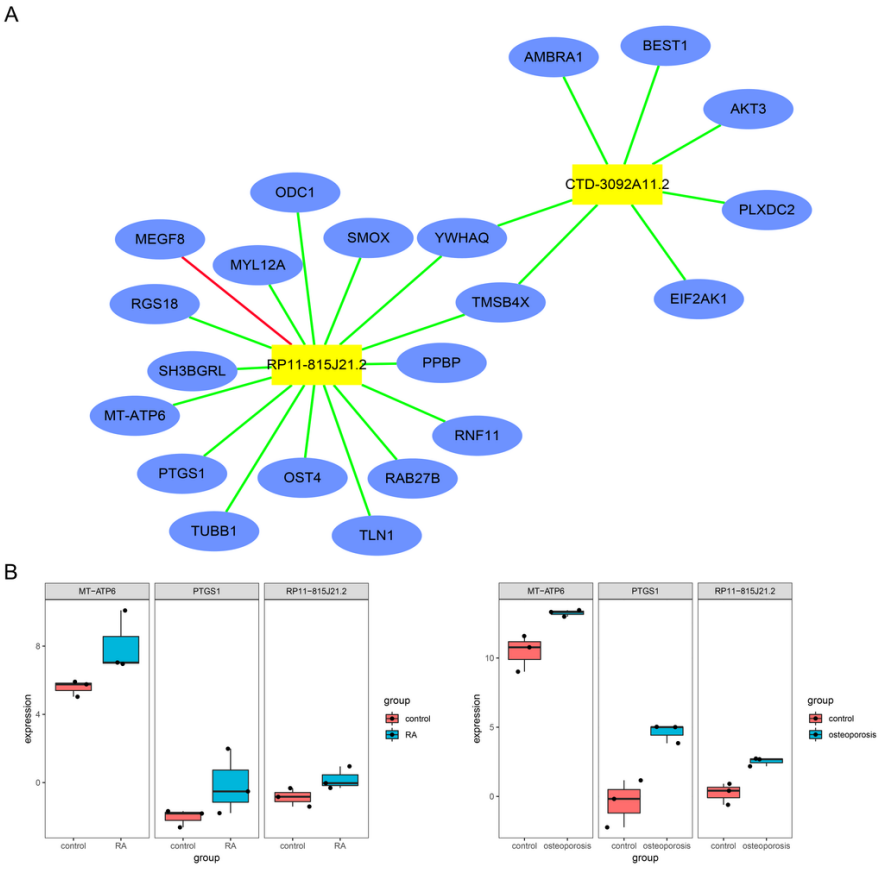

Figure 6.Co-expression network between common DEmRNAs and DElncRNAs. (A) Co-expression network showed the interaction between mRNAs and lncRNAs.Blue ellipse nodes represent mRNAs, yellow square nodes represent lncRNAs. The red line between mRNA and lncRNA indicate a negarive correlation, while the green lines indicate a positive correlation.(B) Box plot showed the expression pattern of two lncRNA-mRNA pairs (RP11−815J21.2 - MT−ATP6 and RP11−815J21.2- PTGS1).

Figure 6

See figure for legend.